PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 341-B0497	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/12514	International filing date *(day/month/year)* 10.11.2003	Priority date *(day/month/year)* 13.11.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48		
Applicant BRACCO IMAGING SpA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.05.2004	07.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Vadot, P Telephone No. +31 70 340-3968

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/12514

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-25 as originally filed

Claims, Numbers

1-22 as originally filed

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-22 (all partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-22 (all partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-22 (all partially)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22 (all partially)
Industrial applicability (IA)	Yes:	Claims	1-22 (all partially)
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

No International Preliminary Examination will be carried out in respect of subject-matter which is not covered by the search report (Rule 66.1 (e) PCT).

Re Item V

Reasoned statement under Art. 35.2 PCT with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1 Reference is made to the following documents:

D1: ARTEAGA DE MURPHY C ET AL: 'PHOSPHINE REDUCED IGG: A NEW METHOD FOR 99MTC LABELING IMMUNOGLOBULINS' JOURNAL OF RADIOANALYTICAL AND NUCLEAR CHEMISTRY, ARTICLES, ELSEVIER SEQUOIA S.A., LAUSANNE, CH, vol. 220, no. 1, 1997, pages 41-45, XP000199389

D2: BURNS J A ET AL: 'Selective reduction of disulfides by tris(2-carboxyethyl)phosphine' JOURNAL OF ORGANIC CHEMISTRY,AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 56, no. 8, 1991,pages 2648-2650, XP002149302 ISSN: 0022-3263

D3: SINGH RAJEEVA ET AL.: 'Labeling of antibodies by in situ modification of thiol groups generated from selenol-catalyzed reduction of native disulfide bonds.' ANALYTICAL BIOCHEMISTRY, vol. 304, - 2002 pages 147-156, XP001165655

D4: SEITZ U ET AL: 'PREPARATION AND EVALUATION OF THE RHENIUM-188-LABELLED ANTI-NCA ANTIGEN MONOCLONAL ANTIBODY BW 250/183 FOR RADIOIMMUNOTHERAPY OF LEUKAEMIA' EUROPEAN JOURNAL OF NUCLEAR MEDICINE, BERLIN, DE, vol. 26, no. 10, October 1999 (1999-10), pages 1265-1273, XP000952569 ISSN: 0340-6997

D5: WO 91 04056 A (IMMUNOMEDICS INC) 4 April 1991 (1991-04-04)

5.2 Novelty:
The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-22 is not new in the sense of Article 33(2) PCT.

Document D1 deals with the reduction of IgG by a TCEP reducing treatment. The reduced antibody is then labelled by 99m-Tc.

Document D3 discloses Fab fragments manufactured by reduction of inter- and intra- disulfide bonds, with TCEP. These fragments are then biotinylated.

Document D4 discloses the preparation of reduced antibodies by the use of TCEP. These antibodies are then radiolabeled by rhenium-188. It clearly states the advantage of TCEP over the other known reducing agents. The number of SH groups generated using this method of reduction, is 6.7+-0.7 SH per antibody.

5.3 Inventive step:
Even if novelty were to be established, the subject matter of claims 1-22 does not meet the criteria of Article 33(3)PCT with regard to inventive step.

Document D5 claims the use of Fab fragment having free sulfhydryl group by reduction of disulfide bonds. This Fab fragment is then labelled with Technetium.

This last document is considered to be the closest prior art.

The difference between D5 and the application under examination is the use of TCEP as reducing agent for a selective and quantitative reduction of the inter-chain.

D2 explains the selective reduction of disulfide bonds by tris(2-carboxyethyl)phosphine.

The problem underlying the application is to selectively reduce disulfide bonds of a Fab fragment in order to conjugate it to molecular entities (viz. diagnostic or therapeutic utility), via a spacer arm, as for instance a maleimido group.

Starting from D5 and confronted with the problem hereabove, the skilled person would certainly use the teachings of D2, thus arriving at the presently claimed invention.